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Reproductive hormonal profile and estrous cycles of gilts fed fermented and enzyme-supplemented cassava peels meal based diets

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Abstract; *Fresh cassava peels were collected, fermented for four days and sundried for 3-5 days. It was ground and used to compound maize replaced grower and finisher diets. A group of 27 weaner gilts (Largewhite x Duroc), aged 8 weeks and weighed 10.61±0.27kg were fed the diets. The weaner gilts were allotted to three treatments comprising T_1 (control), T_2 (fermented CPM) and T_3 (fermented CPM + maxigrain^R enzyme) in a completely randomized design. The diets were given at 4% of their live body weights daily throughout the experiment. At 20 weeks of age, three gilts per treatment were selected and watched for symptoms of heat which were scored using a scale of 1 - 4. In a similar vein, the gilts were monitored for expression of heat, which was recorded. Each was bled of 3-5ml fresh blood in the 3rd day of third estrous cycle via venopuncture every thirty minutes for six hours and was transferred into clean and sterile test tubes, allowed to coagulate, serum decanted and stored at -20°C for hormonal assays of estradiol 17β, follicle stimulating hormone (FSH) and leuteinizing hormone (LH). Data were collected and analysed using one way analysis of variance and difference in means separated with Duncan's Multiple Range Test. The results showed that the duration of estrous cycle was not significantly different ($p>0.05$) and spanned between 20.33 - 21.33, 19.33 – 20.33 and 19.33 – 20.00 days for T_1, T_2 and T_3 respectyively. Estradiol-17β ranged from 7.28 – 12.73, 7.14 – 11.26 and 7.62 – 8.86 pg/ml for T_1, T_2 and T_3 respectively and showed significant difference ($p<0.05$) during 2nd, 3rd and 5th hours. The LH and FSH had no significant difference ($p>0.05$), however, ranged from 3.72 – 14.38, 3.53 – 13.22 and 3.92 – 15.86 ng/ml and 1.72 – 8.43, 1.63 – 8.88 and 1.61 – 8.94 ng/ml for T_1, T_2 and T_3 respectively. Therefore, fermented cassava peels with or without enzyme supplementation has no deleterious effect on the external estrus symptoms and reproductive hormones.*

Keywords*: Gilts, fermented cassava peels, maxigrain^R enzyme, oestrous cycle, reproductive hormones*

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I. Introduction

A significant increase in the growth of follicles selected for ovulation seems to occur on days 14-17 of the estrous cycle (Foxcroft and Hunter, 1985; Ryan et al., 1994; Cox, 1997). Ryan et al., (1994) reported that the number of follicles increased from approximately 20 at day 4 to 45 during 16-17th day of the estrous cycle. The percentage of large follicles greater than 5mm increased from 6% at days 16-19 to 12% at day 20. According to Knox (2005) in gilts, the number of large follicles greater than 6.9mm started to increase on day 15 of the estrous cycle, reaching a maximum during the first day of estrus (day 0). During the same period, the number of small and medium-sized follicles was decreasing (Knox, 2005).

Hasegawa et al. (1988) reported that concentrations of serum inhibin revealed two small peaks during the luteal phase and a large plateau peak, which reached its maximum four days before ovulation during the estrous cycle in sows. The maximum concentration of inhibin coincided with the lowest concentration of FSH. Knox et al. (2003) found that the concentration of FSH started decreasing from around day 15 of the normal estrous cycle ie with 19-21 days inter-estrous intervals. Gilts expressing high ovulation rate had a significantly higher plasma concentration of FSH than gilts with low ovulation rate during the ovulatory period and the luteal phase of estrous cycle. Concentrations of inhibin ∝-subunit were higher in highly ovulating gilts than in gilts with low ovulation rate during entire estrous cycle (Knox et al., 2003). Increase in ovulation rate did not influence the secretion of estradiol, LH and progesterone (Madej et al., 2009). Evidence is accumulating that FSH alone is not able to stimulate the growth of large follicles or even estradiol secretion in gilts (Guthrie,

2005). The only way to increase ovulation rate in gilts is to feed animals with a modified diet (Madej et al., 2009).

Ferguson et al. (2003) reported that feeding the high volume diet of 3.5kg per day for 19 days resulted in a significantly higher number of LH pulses per 8hr during both luteal and follicular phases of estrous cycle compared with gilts fed maintenance diet of 1.35kg. Once gilts reach puberty at 6-8 months of age, they display estrus at 18-22 day intervals unless cycling is interrupted by pregnancy and lactation, poor nutrition or disease. During this period, gonadotropin releasing hormone (GnRH) is released from the hypothalamus and travels through the blood vessels to the pituitary gland where it stimulates secretion of FSH and LH (Glen, 2009). During the 2-3 day period, just prior to estrus, increasing blood levels of FSH and LH cause follicles to rapidly grow on each of the two ovaries. These follicles secrete increased levels of the hormone estrogen into circulation, which in turn causes the behavioral and physiological changes associated with estrus (eg reddening and swelling of the vulva, lordosis or ''standing response'' in the presence of a boar etc). Rising concentrations of estrogen eventually triggers increased secretion of GnRH, resulting in a massive release of LH during estrus (Glen, 2009). LH peak occurred up to 6hr after GnRH in gilts (Brussow et al., 1993; Brussow et al., 1994) and sows (George et al., 1989; Ogasa et al., 1991). The peak height of LH surge ranged from 4.2 to 16.0 ng/ml (Brussow et al., 1993; Brussow et al., 1994; Parvizi et al., 1976; Enne et al., 1981; Redmer and Day, 1981; Ziecik et al., 1982; Egerszegi et al., 2003). This study was aimed at evaluating the effect of fermented cassava peel meals on exhibition of external estrus symptoms and the consequence, if any, on the expression and magnitude of reproductive hormones in gilts fed diets compounded with such treated CPM.

II. Materials and Methods

Experimental site

The experiment was carried out at the piggery unit of The Research Farm of The Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State, Nigeria. Ibadan is geographically located at latitude 7° 22' 39'' N and longitude 3° 54' 21'' E. Ibadan has a tropical wet and dry climate, with a lengthy wet season. It has mean total rainfall of 1420.06 mm, mean maximum and minimum temperatures of 26.46 °C and 21.42 °C respectively and relative humidity of 74.55%.

Source of ingredients and feed formulation

Fresh cassava peels for this experiment was sourced from Orile-Ilegun; an industrial layout in Ibadan, Oyo State, Nigeria. The maxigrain^{R} enzyme was sourced from open market with the following constituents: Amylase, xylanase, Beta-glucanase, cellulose, pectinase, protease, phytase and lipase. Four processing methods were carried out on cassava peels to determine the process that would reduce hydrogen cyanide glycoside most, improve crude protein content and being more practicable by the rural farmers. Such processing method (fermentation) was used to treat the cassava peels for onward compounding of the experimental diets thus:-

T_1 = Conventional maize-based diet (control).

T_2= Diet with 40% maize-replaced fermented cassava peels.

T_3=Diet with 40% maize-replaced fermented cassava peels supplemented with maxigrain^{R} enzyme.

Experimental animals, design, management and duration

A group of 27 female weaner pigs (Largewhite x Duroc), aged 8 weeks and weighed 10.61±0.27kg each, with good body condition, conformation and at least six pairs of teats, were used for this experiment. Measurement and recording of their body weights were carried out using weighing balance and were allotted to the above treatments using completely randomized design. Each treatment was replicated thrice. Close observations for deformity and other aberrations that could render them unfit for the experiment were looked out for and replacements made in their eventuality. The pigs were also prophylactically taken care of against endo- and ectoparasites using ivomec^{R} (ivermectin) injection at the dose of 1ml/33kg body weight, subcutaneously. There was also administration of long acting oxytetracycline injection at the dose rate of 1ml/10kg body weight (i/m) which was repeated after 72hours to help eliminate possible pathogenic microbes that had not manifested as disease(s). Grower diet (Table 1) was introduced at 4% of their body weight daily (Santiago and Tegbe, 1987; Onyimonyi, 2002). The grower diets were given for the first eleven weeks after which they were replaced with finisher diet (Table 2) till the end of the experiment. Clean drinking water sourced from the borehole in the farm was supplied ad-libitum to the pigs.

Experimental procedure

At 20 weeks of age (5 months), the selected gilts were watched out on daily basis for symptoms of heat. The symptoms involved the exhibition of grunting, restlessness, running after and attempting to mount pen mates, redness and oedema of vulva and release of whitish discharge from the vulva. The degree of expression of heat was evaluated according to the following scale, which ranges from 1 to 4, when the heat symptoms are:

1= so vague that the observer will not completely be assured that the gilt is on heat,
2= so weaker than usual, but the observer is not in doubt as to whether or not the gilt is on heat,
3= of normal strength and,
4= more evident than normally.
(Rottensten and Touchberry, 1957)

Reproductive hormone assays

Three gilts from each treatment group that showed symptoms of estrus were selected for the trial on the 3rd day of estrus during the third estrous cycle. They were bled of 3-5ml of fresh blood via venipuncture every thirty minutes (30 minutes) for six hours (between 8 am and 1 pm) according to the method of Anne-Marie (1987) and Helene et al. (2000). The blood was transferred into clean and sterile test tubes where coagulation took place within ten minutes and serum was decanted and stored at -20°C for hormonal assays of estradiol 17β, follicle stimulating hormone (FSH) and leuteinizing hormone (LH) using the method of Helene et al. (2000) and Tomic et al. (2007).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and means with statistical difference separated using Duncan's Multiple Range Test (Duncan, 1955).

III. Results And Discussions

Reproductive hormonal profiles and oestrous cycles of the pigs

In this study, the range of LH (Table 3) was 3.53 –15.86 ng/ml which is similar to the findings of Egerszegi et al. [2003] who recorded 4.2–16 ng/ml in landrace sows. The values were also within the range observed in spontaneous or synchronized estrous gilts [Parvizi et al., 1976; Enne et al., 1981; Redmer and Day, 1981; Ziecik et al., 1982; Brussow et al., 1993; Brussow et al., 1994]. This observation shows that the energy and protein contents of the treatment diets were adequate and that treatment of CPM through fermentation and maxigrain^R enzyme supplementation were remedial to HCN content associated with cassava peels.

By Table 4, the range of estradiol 17β [7.14–12.73pg/ml] in this study was lower than the findings of Vande-Wiel et al. [1981] who got maximum concentration of between 32.1 and 56.4pg/ml but reported it as occurring at 8 – 15 hours before the time of LH maximum surge whereas Enne et al. [1981] reported the same at 24 hrs. The variation between the present study and that of earlier reports as above could be due to timing, since surge of estradiol occured before surge of LH and after which, there was a gradual decrease of estradiol to enhance ovulation by LH surge. This study was carried out during the influence of LH surge (periovulatory period). FSH dominance is usually challenged by a concomitant increase in the concentrations of estradiol during proestrus and this indicated follicular development [Lucy et al., 2001]. The FSH as in Table 5 was not at its maximal concentrations [1.61 – 9.21ng/ml] since it was determined during or very near to estrus. FSH initially predominated, resulting in follicular growth and maturation, after which it receded near estrus. Table 6 shows the lengths of the estrous cycles in this study which ranged from 19.33 to 21.33 days. Many authors have reported similar estrous cycles (Singleton and Diekman, 1990; Tur, 2013; Motaleb et al., 2014; Sinha et al., 2015).

IV. Conclusion

Fermented cassava peels with or without enzyme supplementation has no deleterious effect on the oestrous cycle and reproductive hormonal expression.

Table 1:- Dietary composition of pig's grower diets

	T_1	T_2	T_3
Maize	40.00	-	-
CPM	-	40.00	40.00
PKC	20.00	29.50	29.50
BDG	14.00	10.00	10.00
GNC	12.50	11.00	11.00
BLM	5.00	5.00	5.00

Palm oil	4.00	4.00	4.00
Bone meal	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00
Methionine	0.20	0.20	0.20
Lysine	0.75	0.75	0.75
Premix	0.40	0.40	0.40
Salt	0.15	0.15	0.15
Total	100	100	100
C.P. (%)	20.82	20.47	20.47
ME (kcal/kg)	2871.50	2759.23	2759.23

Table 2:- Dietary composition of pig's finisher diets

	T_1	T_2	T_3
Maize	40.00	-	-
CPM	-	40.00	40.00
PKC	22.50	23.50	23.50
BDG	10.00	10.00	10.00
W/O	14	9.00	9.00
SBM	-	4.00	4.00
BLM	5.00	5.00	5.00
Palm oil	4.00	4.00	4.00
Bone meal	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00
Methionine	0.20	0.20	0.20
Lysine	0.75	0.75	0.75
Premix	0.40	0.40	0.40
Salt	0.15	0.15	0.15
Total	100	100	100
C.P. (%)	17.02	17.36	17.36
ME (kcal/kg)	2822.88	2710.23	2710.23

Provided the following/kg diet:Vitamin A–8,000 IU, Vitamins D3 –3,000 IU, Vitamins E–8 IU, Vitamin K – 2mg, Vitamin B1– 1 mg, Vitamin B2–0.2 mg, Vitamin B12–5 mg, Nicotinamide –10 mg, Selenium– 0.1 mg, Ca Pantothenate – 5 mg, Folic acid –0.5 mg, Choline Chloride –150 mg, Iron –20 mg, Manganese –80 mg, Copper –8 mg, Zinc –50 mg, Cobalt –0.225mg, Iodine –2 mg Antioxidant – 0.1ppm

Key:- CPM = Cassava peels meal, PKC = Palm kernel cake, W/O = Wheat offal, BDG = Brewer's dried grain, SBM = Soybean meal, BLM = Blood meal, C.P. = Crude protein, ME = Metabolizable energy.

Table 3: Profile of Leuteinizing Hormone (ng/ml) of pigs fed CPM-based diets

Time (hr)	T_1(control)	T_2(CPM only)	T_3(CPM + Enzyme)
0	4.92±0.04	4.46±0.13	4.96±0.06
0.5	5.73±0.02	5.73±0.09	5.73±0.09
1	6.03±0.05	6.27±0.17	5.62±0.23
1.5	5.93±0.06	6.41±0.07	6.82±0.16
2	5.65±0.04	5.94±0.08	6.43±0.05
2.5	7.78±0.06	6.88±0.04	7.97±0.24
3	14.38±0.13	13.22±0.07	15.86±0.14
3.5	11.57±0.21	13.81±0,11	14.37±0.12
4	3.72±0.15	3.53±0.14	3.92±0.04
4.5	4.18±0.22	4.31±0.06	5.08±0.21
5	4.46±0.14	4.61±0.14	4.31±0.11
5.5	4.87±0.17	5.11±0.04	4.17±0.06
6	12.41±0.07	12.36±0.21	14.44±0.25

Table 4: Estradiol 17β (pg/ml) profile of pigs fed CPM-based diets

Time (hr)	T_1(control)	T_2(CPM only)	T_3(CPM + Enzyme)
0	8.63±0.06	7.14±0.07	8.86±0.10
1	8.84±0.09	7.33±0.04	7.92±0.08
2	$7.28±0.08^b$	$11.26±0.05^a$	$8.72±0.06^b$
3	$7.54±0.08^b$	$9.21±0.06^a$	$7.97±0.11^b$
4	8.36±0.06	7.38±0.05	7.43±0.07
5	$12.73±0.10^a$	$9.12±0.13^b$	$8.04±0.04^b$
6	8.49±0.09	8.78±0.05	7.62±0.07

ab:- means on the same row with different superscripts are statistically different ($p<0.05$)

Table 5: Profile of Follicle Stimulating Hormone (ng/ml) of pigs fed CPM-based diets

Time (hr)	T_1(control)	T_2(CPM only)	T_3(CPM + Enzyme)
0	1.72±0.12	2.23±0.09	2.08±0.15
0.5	2.52±0.09	2.88±0.11	2.11±0.13
1	1.88±0.07	2.18±0.08	2.58±0.08
1.5	2.75±0.15	3.22±0.07	3.44±0.21
2	2.16±0.14	2.09±0.13	3.31±0.17
2.5	3.47±0.06	3.41±0.09	3.68±0.07
3	8.32±0.17	8.88±0.06	8.94±0.16
3.5	8.43±0.03	9.16±0.15	8.89±0.18
4	8.37±0.07	8.41±0.12	9.21±0.15
4.5	1.86±0.04	1.72±0.08	1.61±0.05
5	2.06±0.08	1.63±0.19	2.33±0.16
5.5	4.32±0.12	4.68±0.21	4.57±0.08
6	7.82±0.08	8.46±0.16	8.22±0.05

Table 6: Duration of estrous cycles (days) in pigs fed CPM-based diets

Heat	T_1(control)	T_2(CPM only)	T_3(CPM + Enzyme)
First heat (days)	20.33±0.67	20.33±0.33	21.33±0.33
Second heat (days)	20.33±0.33	19.33±0.33	20.33±0.33
Third heat (days)	19.33±0.33	20.00±0.01	20.00±0.58

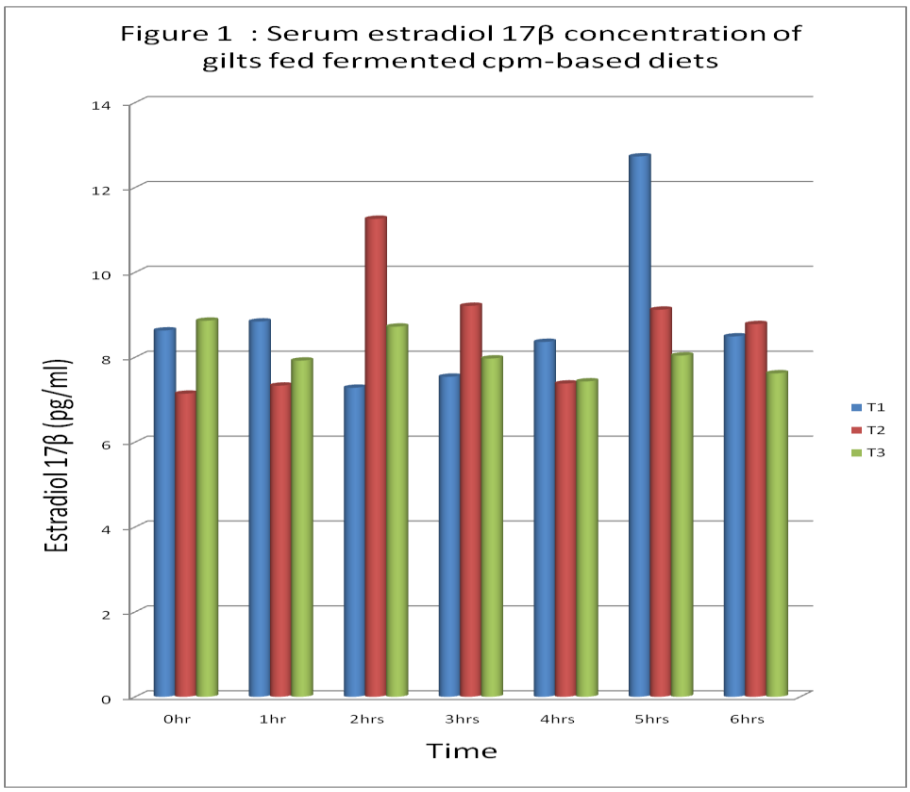


Figure 1 : Serum estradiol 17β concentration of gilts fed fermented cpm-based diets
Estradiol 17β (pg/ml)
14
12
10
8
6
4
2
0
0hr
1hr
2hrs
3hrs
4hrs
5hrs
6hrs
Time
T1
T2
T3

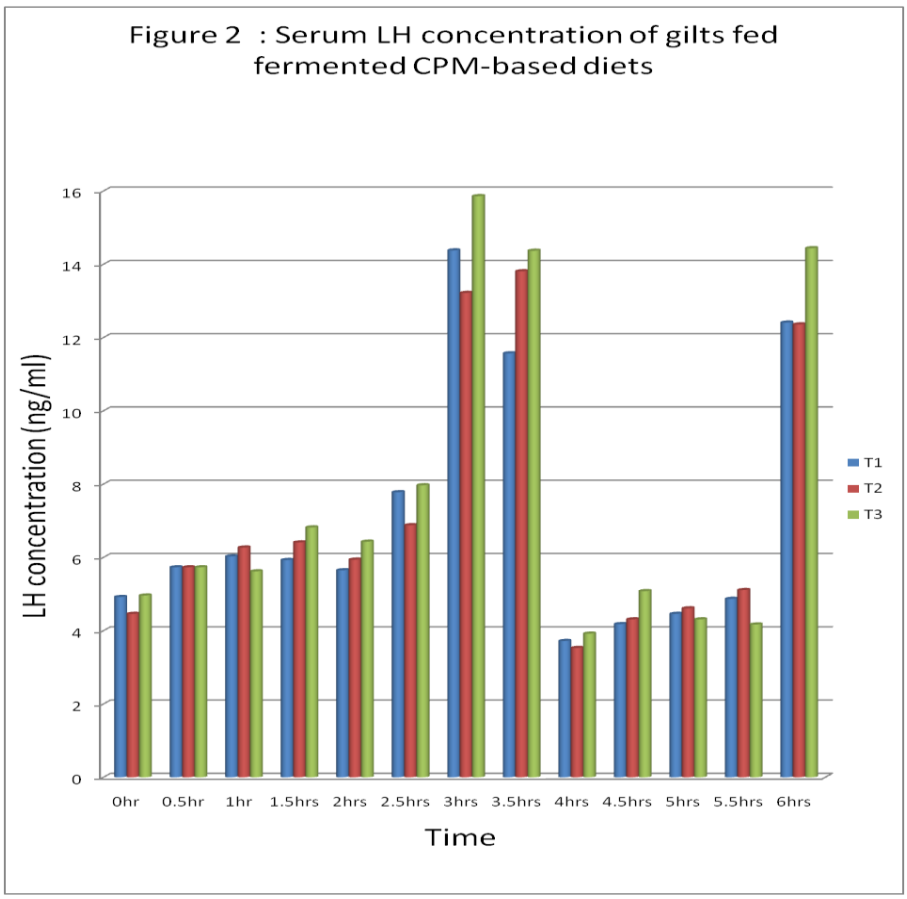
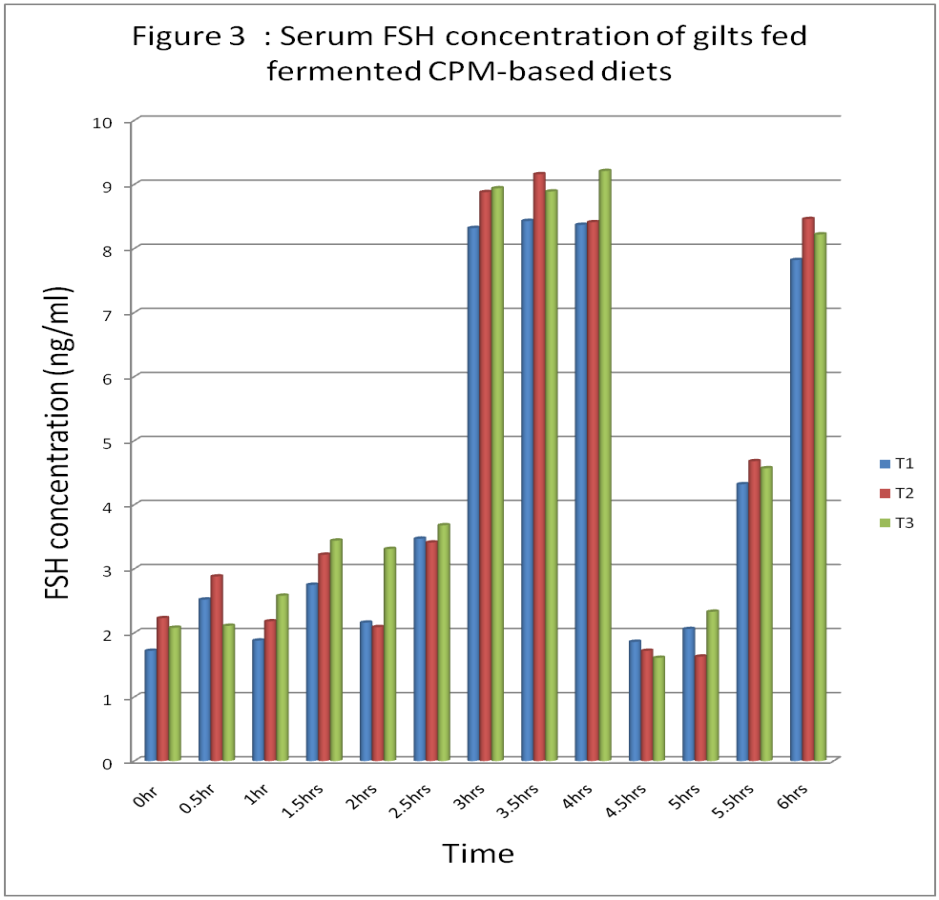


Figure 2 : Serum LH concentration of gilts fed fermented CPM-based diets
LH concentration (ng/ml)
16
14
12
10
8
6
4
2
0
0hr
0.5hr
1hr
1.5hrs
2hrs
2.5hrs
3hrs
3.5hrs
4hrs
4.5hrs
5hrs
5.5hrs
6hrs
Time
T1
T2
T3



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